

Early Serum Galactomannan Trend as a Predictor of Outcome of Invasive Aspergillosis

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The monitoring and prediction of treatment responses to invasive aspergillosis (IA) are difficult. We determined whether serum galactomannan index (GMI) trends early in the course of disease may be useful in predicting eventual clinical outcomes. For the subjects recruited into the multicenter Global Aspergillosis Study, serial GMIs were measured at baseline and at weeks 1, 2, and 4 following antifungal treatment. Clinical response and survival at 12 weeks were the outcome measures. GMI trends were analyzed by using the generalized estimation equation approach. GMI cutoffs were evaluated by using receiver-operating curve analyses incorporating pre- and posttest probabilities. Of the 202 study patients diagnosed with IA, 71 (35.1%) had a baseline GMI of ≥ 0.5 . Week 1 GMI was significantly lower for the eventual responders to treatment at week 12 than for the nonresponders (GMIs of 0.62 ± 0.12 and 1.15 ± 0.22 , respectively; $P = 0.035$). A GMI reduction of $>35\%$ between baseline and week 1 predicted a probability of a satisfactory clinical response. For IA patients with pretreatment GMIs of <0.5 ($n = 131$; 64.9%), GMI ought to remain low during treatment, and a rising absolute GMI to >0.5 at week 2 despite antifungal treatment heralded a poor clinical outcome. Here, every 0.1-unit increase in the GMI between baseline and week 2 increased the likelihood of an unsatisfactory clinical response by 21.6% ($P = 0.018$). In summary, clinical outcomes may be anticipated by charting early GMI trends during the first 2 weeks of antifungal therapy. These findings have significant implications for the management of IA.

Invasive aspergillosis (IA) remains the most common mold infection, causing significant mortality and morbidity in immunocompromised patients (16, 19). Securing a firm diagnosis of the disease and monitoring the response to treatment are difficult, as the patient may not exhibit reliable symptoms and signs in the presence of neutropenia and immune-modulating drugs, such as corticosteroids. Over the years, the diagnostic capability for the detection of IA has improved through the use of *Aspergillus* galactomannan (GM) antigen. GM from the *Aspergillus* cell wall is released during invasive disease, and the level of circulating GM may be indicative of the intrinsic fungal burden in the host, at least in patients with underlying hematological malignancies or undergoing hematopoietic stem cell transplantations (HSCTs) (13). GM can be detected by the Platelia *Aspergillus* enzyme immunoassay (EIA) (Bio-Rad Laboratories), and results are reported as the galactomannan index (GMI) (14, 23). GMI measurements have been studied extensively, gaining general acceptance as a noninvasive diagnostic tool for IA for at-risk patients (9), and have been included as a mycological criterion for the case definition of probable IA by the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) consensus group (2, 7, 20).

Furthermore, in addition to serving as a diagnostic tool, serial GMI determinations have been suggested to be useful for monitoring the treatment response (1) or as a surrogate endpoint for outcomes of invasive aspergillosis (8, 12, 15). However, the poten-

tial use of GMI trends to predict eventual clinical outcomes early in the course of IA has not been well established, although it may have significant implications for patient management.

Here, we report findings from a study involving a cohort of 202 IA patients enrolled in a multicenter antifungal drug trial in which we assessed, retrospectively, whether serial serum GM trends early in the course of treatment correlated with the outcome of IA.

MATERIALS AND METHODS

Study patients and design. Patients had been enrolled in study protocol 150-307, performed in Europe, Israel, and Australia under the aegis of the Invasive Fungal Infections Group of the European Organization for Research and Treatment of Cancer (EORTC). This and study protocol 150-602 comprised the Global Comparative Aspergillosis Study, a multicenter randomized trial that compared the efficacy of voriconazole to that of amphotericin B deoxycholate for the primary treatment of invasive aspergillosis. The selection of eligible patients for the trial as well as the case

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definitions were previously detailed (5). The protocol was approved by the appropriate institutional review boards, and written informed consent was obtained from all patients.

Outcome measures were originally assessed by an independent and blinded data review committee (DRC) based on reviews of clinical, mycological, and systemically collected radiological data (5). A satisfactory clinical response was defined as a complete or partial response at week 12 after the commencement of antifungal therapy, while a poor response was defined as treatment failure or stable disease at the above-described preset endpoint, in accordance with the preestablished assessment criteria of the blinded DRC. The other definitive outcome measure was survival at 12 weeks after the start of antifungal treatment.

Blood samples were obtained serially from trial patients at baseline prior to the initiation of the designated study antifungal therapy and at intervals of weeks 1, 2, and 4 following the initiation of treatment. The specimens were stored at -20°C prior to assays, and multiple freeze-thaw cycles were avoided. Serum GM concentrations were measured at a central laboratory (Health Protection Agency Mycology Reference Laboratory, Bristol, United Kingdom) within 7 to 12 months of the completion of the trial and were performed according to the manufacturer's instructions (Platelia *Aspergillus* EIA; Bio-Rad Laboratories, Marne-la-Coquette, France). Results were recorded as the galactomannan index (GMI), which was relative to the optical density of the mean cutoff of the control sample obtained in the same run. Random testing for the stability of the stored specimens over a maximum period of 13 months yielded an average GMI decrease of 11%. All reagents were obtained from Bio-Rad Laboratories. The GM results were not included in the primary analysis, as the measurements were performed after efficacy and safety data had been reported (5). For the purpose of this study, cases have been reclassified according to the 2008 EORTC/MSG criteria for invasive aspergillosis (2). Galactomannan results have been incorporated for this classification.

Statistical analysis. The time profiles of the GMI or ΔGMI (GMI change between 2 specified time points) for different outcomes at week 12 (based on the clinical response and survival at week 12) were presented graphically by plotting the mean per time point with the standard error of the mean (SEM). The generalized estimation equation (GEE) approach was used to test and estimate changes in the effect size for the GMI, accounting for repeated measurements over time within patients and adjusting for the following covariates: age, sex, underlying hematological condition (leukemia/lymphoma versus hematopoietic stem cell transplantation), neutropenia, and receipt of the primary trial drug (amphotericin B versus voriconazole). Neutropenia was defined as a neutrophil count of less than 500 per cubic millimeter in the previous 2 weeks prior to recruitment into the trial. The generalized linear model (GLM) using the binomial family and identity link function was used to estimate the risk differences in the magnitude of the ΔGMI and to ascertain the differences in the effect size in relation to the specified clinical outcome. The above-described covariates were factored into the regression analyses.

Results from the trend analysis using the GEE approach were used to indicate which GMI study parameters might potentially be of use as a predictive tool. The positive likelihood ratio (LHR) was used to assess the utility of the GMI as a prognostic test. The conditional probability of the LHR was calculated as $[(T^+ C^+)/\text{total } C^+]/[(T^+ C^-)/\text{total } C^-]$, where T^+ is test positivity and C^+ or C^- denotes the presence or absence of a condition, respectively (i.e., clinical response or survival by week 12) (3). An LHR of >1.0 was taken as a positive test result to predict the probability of the designated condition/outcome. Receiver-operating characteristic (ROC) analyses were performed against the selected GMI study parameter, which may be either absolute GMI values at the specified time point or based on the change of the GMI between 2 specified time points, i.e., the ΔGMI , to examine the predictive abilities of different cutoff points for GMI values. The threshold for statistical significance was set at a P value of <0.05 for the above-mentioned statistics.

TABLE 1 Demographic characteristics of the study cohort and the treatment-related outcomes of interest^a

Characteristic	No. (%) of patients ($n = 202$)		P value
	Baseline GMI ≥ 0.5 ($n = 71$)	Baseline GMI < 0.5 ($n = 131$)	
Sex			
Male	46 (64.8)	83 (63.4)	0.84
Female	25 (35.2)	48 (36.6)	
Certainty of disease			
Proven IA	5 (7.0)	12 (9.1)	<0.001
Probable IA	66 (93.0)	64 (48.9)	
Possible IA	0 (0)	55 (42.0)	
Pulmonary IA	62 (87.3)	121 (92.4)	0.24
Neutropenia	49 (69.0)	86 (65.7)	0.63
Underlying diseases			
Leukemia/lymphoma	51 (77.3)	102 (81.0)	0.55
HSCT	15 (22.7)	24 (19.0)	
Antifungal treatment			
Voriconazole	40 (56.3)	63 (48.1)	0.26
Amphotericin B	31 (43.7)	68 (51.9)	
Wk 12			
Responders	29 (40.9)	65 (49.6)	0.23
Nonresponders	42 (59.1)	66 (50.4)	
Wk 12			
Survivors	48 (67.6)	97 (74.1)	0.33
Nonsurvivors	23 (32.4)	34 (25.9)	

^a HSCT, hematopoietic stem cell transplantation. The week 12 response is the clinical response at week 12, as assessed by the data review committee (DRC).

RESULTS

Two hundred two patients had a diagnosis of proven, probable, or possible invasive aspergillosis, with at least two serum GMI results available for analysis. The patient demographics are presented in Table 1. Of these patients, 183 (90.6%) had invasive pulmonary aspergillosis. One hundred fifty-three patients (75.7%) had underlying leukemia or lymphoma and were receiving chemotherapy, while another 39 (19.3%) were HSCT patients.

Serum galactomannan measurements were performed on 695 distinct serial specimens obtained at the respective study intervals: baseline ($n = 202$) and week 1 ($n = 179$), week 2 ($n = 165$), and week 4 ($n = 149$) after the initiation of antifungal therapy. Seventy-one of the 202 patients in the trial (35.1%) had a baseline GMI measurement of ≥ 0.5 .

Serial GMIs at baseline and at weeks 1, 2, and 4 were assessed in relation to the eventual clinical response and survival at week 12. The GMIs generally showed a downward trend with anti-*Aspergillus* treatment. Notably, GMI trends of the proven/probable IA subcohort ($n = 147$) were similar to those of the overall IA patient cohort of the original primary efficacy study (5) (consisting of proven, probable, or possible IA cases [$n = 202$] [see Fig. S1 in the supplemental material]), and this larger cohort was employed for the intent of this study. In lieu of the Platelia *Aspergillus* EIA diag-

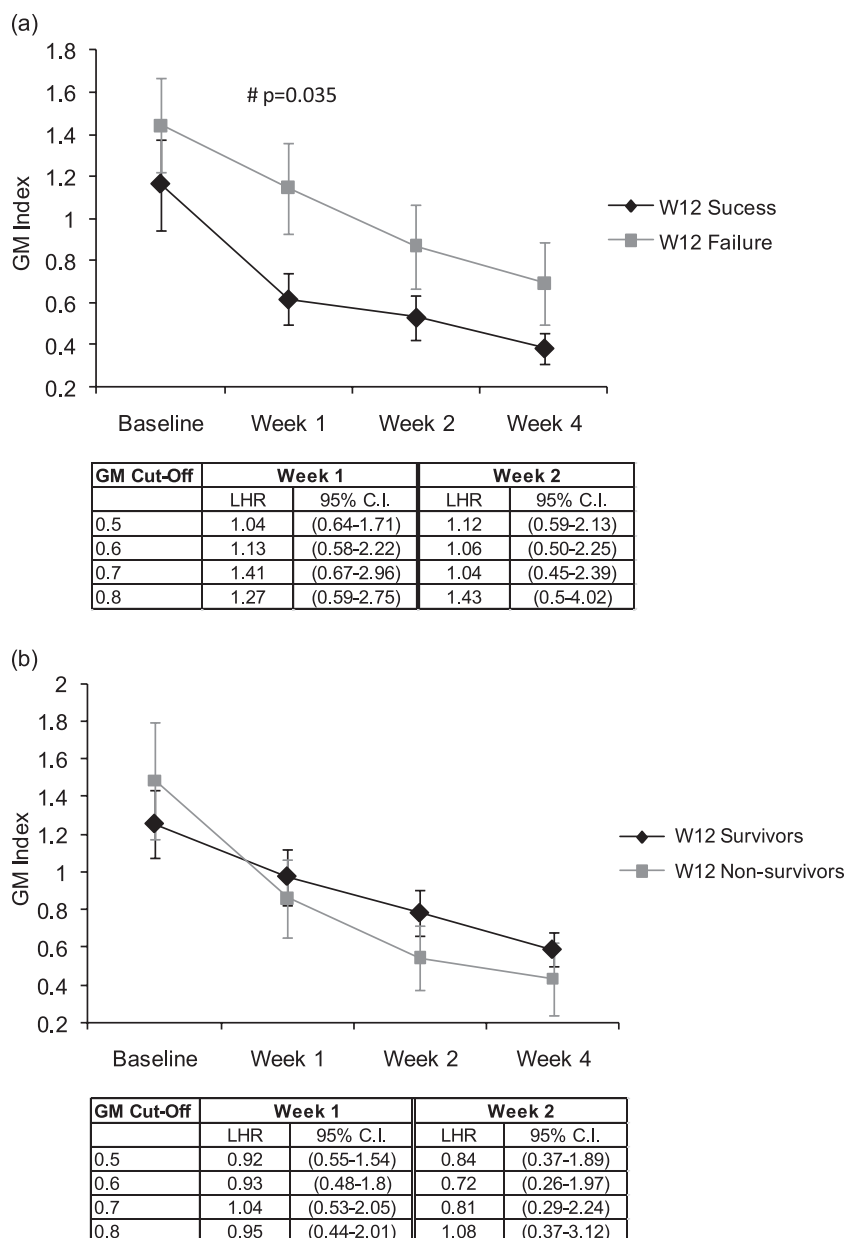


FIG. 1 Galactomannan index (GMI) of patients with a positive GMI of ≥ 0.5 at baseline plotted against predefined clinical outcome parameters at week 12: treatment response (a) or survival (b). # p is the P value for the difference in the absolute GMI values between W12 responders and nonresponders. This was 0.003 by univariate analysis. After multivariate correction for age, sex, underlying hematological condition, neutropenia, and receipt of the primary trial drug (amphotericin B or voriconazole), # p was 0.035. The numbers of distinct specimens analyzed at each time point are as follows: 71 specimens at baseline, 60 at week 1, 53 at week 2, and 42 at week 4. Statistical analysis was performed by the generalized estimation equation (GEE) approach. The table below each graph depicts positive likelihood ratios (LHR) and 95% confidence intervals (95% C.I.) of the respective adverse outcomes (poor clinical response or mortality at week 12) should the GM index remain higher than the cutoff value, as indicated.

nostic cutoff, which is currently established at 0.5 for test positivity (11), we studied the patients stratified as per-baseline pretreatment GMIs of ≥ 0.5 and < 0.5 , as it was observed that the GMI trends differed during the course of therapy, as described below.

GMI and clinical outcomes for patients with baseline GMIs of ≥ 0.5 . Of the 71 IA patients who had a GMI of ≥ 0.5 upon entry into the clinical trial, 29 (40.9%) patients had a satisfactory clinical response at week 12. The remaining 42 (57.1%) patients were assessed to have a poor response to therapy at the end of the study, as determined by the DRC.

(i) Week 12 clinical response. Nonresponders at week 12 (W12 nonresponders) tended to have higher GMIs (mean, 1.44 ± 0.22) at baseline than responders (mean GMI, 1.16 ± 0.22), but this difference was not significant ($P = 0.52$). However, responders at week 12 (W12 responders) had reduced week 1 GMI values compared to those of patients who eventually failed treatment (GMIs of 0.62 ± 0.12 and 1.15 ± 0.22 , respectively; $P = 0.003$ by GEE analysis) (Fig. 1a). This difference remained statistically significant after adjustment for the covariates age, sex, neutropenia, underlying disease, and primary trial drug ($P = 0.035$ by multivariate GEE analysis).

The absolute GMI readout at week 1 was a suboptimal tool to predict clinical responses at 12 weeks (area under the concentration-time curve [AUC] of 0.41 by ROC analysis) despite this difference being statistically significant by GMI profile analysis as described above. The persistence of an absolute GMI of >0.5 at weeks 1 and 2 despite treatment was also not predictive of the eventual clinical response (positive LHR not significantly more than 1) (Fig. 1a). On the other hand, through ROC analysis, the relative change in the GMI from baseline to week 1 ($\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W1}}$) was helpful in predicting the W12 response to IA treatment. A reduction of the GMI of $>35\%$ from baseline to week 1 yielded an LHR of 3.84 for the W12 clinical response (AUC of 0.72). In the context of this specific patient cohort, a $\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W1}}$ value showing a reduction of $>35\%$ following the commencement of IA treatment improved the *post hoc* probability of the clinical response at 12 weeks from 40.9% (at diagnosis of disease) to 72.7% (posttest probability).

(ii) Week 12 survival. Forty-eight of these 71 patients (67.6%) with a baseline GMI of ≥ 0.5 survived until the designated study endpoint. However, GMI trends were nondiscriminative between eventual survivors and nonsurvivors of this cohort (Fig. 1b).

In summary, the above-described results suggest that for IA patients with a baseline GMI of ≥ 0.5 , a significant drop of serial GMI levels during the early course of antifungal treatment is predictive of an eventual satisfactory clinical response. For our patients, a $\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W1}}$ reduction of more than 35% increased the *post hoc* probability of an eventual clinical response to treatment at 12 weeks.

GMI and clinical outcomes for patients with baseline GMIs of <0.5 . One hundred thirty-one patients diagnosed with IA had a baseline GMI of less than 0.5 (2). Sixty-five of these patients (49.6%) had a satisfactory response at week 12 (Table 1). The survival rate was 74.1% for this cohort.

(i) Week 12 clinical response. Baseline and week 1 absolute GMI values were similar in W12 responders and nonresponders with pretreatment GMIs of <0.5 , but notably, a distinct rise in the GMI was seen for W12 nonresponders by week 2 (Fig. 2a). Nonresponding patients displayed a markedly increased $\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W2}}$ (the *P* value was 0.001 by GEE univariate analysis and also after incorporating multivariate adjustments). Likewise, the $\Delta\text{GMI}_{\text{W1} \rightarrow \text{W2}}$ was significant (*P* value of 0.04 to 0.022 following multivariate adjustment).

An increase of the absolute GMI to more than 0.5 after 2 weeks of treatment was associated with a trend toward an unsatisfactory treatment response (LHR of 1.18 to 5.62) (Fig. 2a), although this was not statistically significant. The correlation of the ΔGMI with the W12 clinical response had a modest effect (AUC of 0.59); a $\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W2}}$ increase of 0.17 yielded a likelihood ratio of 4.19 for treatment failure at week 12. Using this cutoff for this cohort of patients, the *post hoc* probability of treatment failure was increased from 50.4% (at the commencement of therapy) to 81.0% (posttest probability) when the $\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W2}}$ increased by more than 0.17.

(ii) Week 12 survival. Early GMI trends between W12 survivors and nonsurvivors mirrored that of the treatment response (Fig. 2b): increasing GMI trends by week 2 were predictive of mortality. After multivariate adjustments were made, the difference in GMI trends ($\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W2}}$ and $\Delta\text{GMI}_{\text{W1} \rightarrow \text{W2}}$) remained significant between survivors and nonsurvivors. The *P*

values for both the $\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W2}}$ and $\Delta\text{GMI}_{\text{W1} \rightarrow \text{W2}}$ were both <0.001 (following univariate and multivariate GEE analyses).

The above-described GMI trends relating to W12 survival were of prognostic value. A rising GMI value beyond 0.5 at 2 weeks despite appropriate treatment was predictive of mortality by week 12 for IA patients whose pretreatment GMI was <0.5 (LHR, 4.48 to 11.00) (Fig. 2b). Furthermore, by applying ROC analysis for the $\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W2}}$, an increase of the GMI of >0.13 yielded a likelihood ratio of 6.65 for eventual mortality by week 12 (AUC of 0.70). Applying these values to this study cohort, patients experiencing an increase in the GMI of >0.13 from baseline to week 2 had their posttest probability of W12 mortality increased 2.4-fold, from 25.9% (upon a diagnosis of IA) to 69.9%.

Hence, for an IA patient who started with a baseline GMI of <0.5 , a rising absolute GMI to >0.5 after 2 weeks despite treatment heralded a poor outcome. Results of the GLM analysis to estimate risk differences further concurred with the above-described findings and showed that among patients with baseline GMIs of <0.5 , every magnitude of increase in the GMI from baseline to week 2 ($\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W2}}$) was associated with an increased likelihood of an unsatisfactory clinical response of 21.6% (95% confidence interval, 19.4 to 23.8; *P* = 0.018) at week 12.

DISCUSSION

In the present study, using a well-characterized cohort of IA patients from the Global Aspergillosis Study, we have demonstrated that early serum GMI trends have prognostic value for clinical outcomes. For IA patients starting treatment with a baseline GMI of ≥ 0.5 , a progressive decline in GMI trends all through the first 4 weeks of therapy was reassuring; in particular, a $\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W1}}$ decline of $>35\%$ increased the probability of a satisfactory clinical response. For patients fulfilling the diagnostic criteria for IA but with a baseline GMI of <0.5 , GMI values remaining low through the first 4 weeks of treatment are anticipated to indicate a satisfactory clinical response. However, in the latter subgroup, every 0.1-unit increase in the GMI between the baseline and after 2 weeks of antifungal treatment ($\Delta\text{GMI}_{\text{baseline} \rightarrow \text{W2}}$) increased the likelihood of a poor clinical response by 21.6%.

The clinical response to antifungal treatment in hematological patients with IA may be obscured in the presence of neutropenia, concurrent immunomodulatory drugs, and the compromised immune system of the host. Until recently, assessments of responses have been difficult in the absence of a reliable surrogate marker of the disease state (21). With the introduction of the Platelia *Aspergillus* GM EIA, much interest has been generated in the usefulness of the GM assay as a diagnostic tool for IA in various clinical settings (9, 10, 18, 22). However, the potential role of GM as a marker of disease status has been less studied and derived mostly from monocentric, limited studies. This aspect was first explored by Boutboul et al., who reported that increasing GMI levels were associated with disease progression (1). It was only recently that two independent groups have reported findings that the serum *Aspergillus* GMI correlates with IA outcomes and could potentially serve as a surrogate disease marker. Using survival as a definitive outcome, Anaissie et al. first noted that the normalization of the serum GMI (to <0.5) after treatment was associated with survival (24). Similarly, Maertens et al. showed that the normalization of the GMI (to <0.5) 6 weeks after the start of antifungal therapy correlated strongly with both response and survival (8). Subsequently, Anaissie et al. performed a literature review of pre-

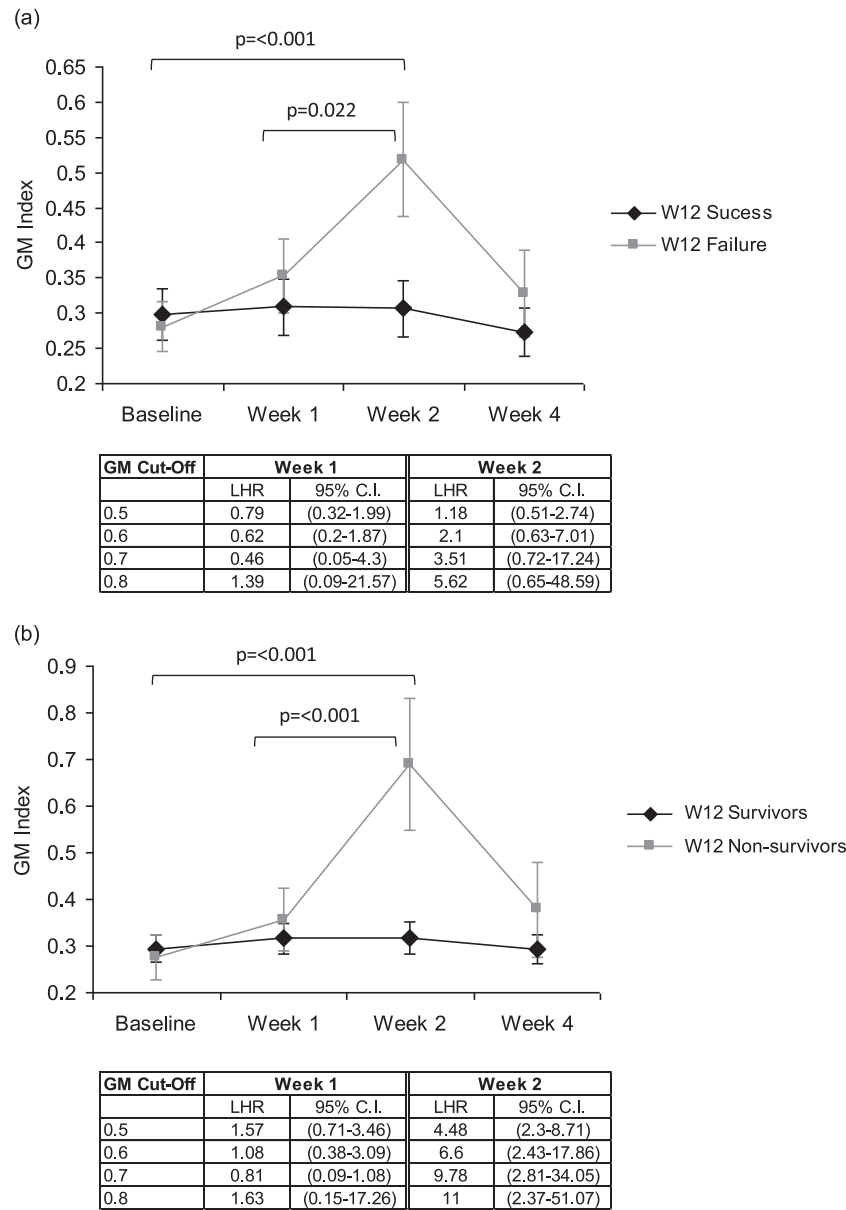


FIG. 2 Galactomannan index (GMI) of patients with a GMI of <0.5 at baseline plotted against predefined clinical outcome parameters at week 12: treatment response (a) or survival (b). The values enclosed by horizontal brackets represent the multivariate P value for the difference in the Δ GMI of both groups between the indicated study intervals, incorporating correction for age, sex, underlying hematological condition, neutropenia, and receipt of primary trial drug (amphotericin B or voriconazole). The numbers of distinct specimens analyzed at each time point are as follows: 131 specimens at baseline, 119 at week 1, 112 at week 2, and 102 at week 4. Statistical analysis was performed by the generalized estimation equation (GEE) approach. The table below each graph depicts the positive likelihood ratios (LHR) and 95% confidence intervals (95% C.I.) of the respective adverse outcomes (poor clinical response or mortality at week 12) should the GM index remain higher than the cutoff value, as indicated.

viously reported studies and found that serial GMI trends through the course of illness showed a strong correlation with clinical outcomes (15). Even more recently, that same group reported that GMI normalization was an early indicator for the assessment of responses (17).

Our findings here differ from the previously reported observations by the above-mentioned latter two groups in three aspects. First, the design of the previous studies stemmed from the original intent of the use of the GMI as a surrogate endpoint to assess outcomes of therapy (12). As such, serial GMI measure-

ments for IA patients were monitored until the eventual outcome as the endpoint (i.e., either discharge or death) by Anaissie et al. (15, 24), or, in the case of Maertens et al., the earliest assessment point was at 6 weeks after the start of antifungal therapy (8). In the latest analysis by Anaissie et al., the study endpoint was the degree of agreement and time to outcome between the EORTC/MSG criteria and GMI normalization. That group found that the time to GMI normalization in clinical responders was in the range of 15 to 41 days (median, 21 days) after the initiation of treatment (17). In our case, we intended to study early GMI trends as a potential

tool to predict eventual clinical outcomes. We were able to demonstrate that GMI trends as early as 1 to 2 weeks after the start of appropriate antifungal therapy might be predictive of the clinical outcome at 12 weeks.

Second, analyses in the previous studies were performed by using the GMI as a dichotomous variable, i.e., either positive with a GMI of ≥ 0.5 or negative with a GMI of < 0.5 . In our study design, we used the GMI as a continuous variable and were able to (i) correlate increasing GMIs with poor outcome/survival and (ii) quantitatively assign an early GMI reduction cutoff value as being associated with a specified eventual clinical response at 12 weeks.

Third, although the GM assay has, to date, been acknowledged as a reliable means to assist the diagnosis of IA (9, 18), we recognize that at the bedside, not all patients with a high suspicion of IA on clinical grounds will fulfill all the EORTC diagnostic criteria (including having a GMI of > 0.5) (2). At-risk patients with a strong clinical suspicion of IA but with GMI of < 0.5 nonetheless will still be initiated on anti-*Aspergillus* therapy by their attending physicians. With our well-characterized cohort of IA patients, we were able to stratify the patients into those with a baseline GMI < 0.5 and those with a baseline GMI of ≥ 0.5 . By monitoring their respective GMI profiles, we also showed that the GMI trends during the course of illness differed between IA patients with starting GMIs of < 0.5 and those with starting GMIs of ≥ 0.5 .

At first sight, our findings here seem analogous to those reported previously by Koo et al. (6), who recently reported that the GMI trend between baseline and week 1 was predictive of mortality at 6 weeks in 74 GM-positive (GMI of > 0.5) IA patients. However, in our patients with baseline GMIs of > 0.5 , the early GMI trend had only a modest prognostic value for clinical outcomes compared with what had been anticipated. On the other hand, in the subcohort of IA patients with pretreatment GMIs of < 0.5 , a rising GMI of > 0.5 by week 2 was linked to adverse outcomes. In addition, we also derived early Δ GMI cutoff thresholds, which were predictive of subsequent adverse clinical outcomes. The use of such simple prediction rules for the clinician at the bedside could enable the timely identification of patients who may potentially fail first-line antifungal treatment, so as to facilitate intervention with more aggressive second-line or combination antifungal therapy.

Invasive aspergillosis remains an invariably complex disease often requiring a protracted treatment course over months. The treatment response over time is determined by a conglomerate of dynamically changing factors, including the status of the primary hematological condition, the reconstitution of the host immune response, the subsequent development of graft-versus-host disease as well as the occurrence of other opportunistic infections, and the institution of concurrent immunosuppressive medication. It is presumptuous to anticipate that early GMI trends over just the first 2 weeks of disease could act as the sole overriding prognosticator of the eventual clinical outcome weeks later. However, our study reveals that initial GMI trends for selected patient cohorts have the propensity to predict not only treatment response but also subsequent mortality. This finding undoubtedly improves our capability to anticipate adverse outcomes for a difficult-to-treat disease, in which the follow-up of the treatment response is also challenging. Nonetheless, it is hoped that as more is known of the pathophysiology of IA, other novel biomarkers and mitigating factors may be incorporated to further improve the current prediction model based on GMI trends.

The robust design of the Global Comparative Aspergillosis Study as well as the sizable patient numbers are pivotal in reinforcing the findings of this study. Of our subjects, 35.1% had a positive GMI of ≥ 0.5 upon the diagnosis of IA. Even for patients with proven and probable IA, the rate of GMI positivity was only 48.3%. The accuracy of the GM assay here was lower than those reported by others in the literature (7, 20). Observations of similarly low GM positivity rates have also been reported by other major centers (4). This unique setting also permitted us to profile the GMI trends of the 2 cohorts (starting GMIs of < 0.5 and ≥ 0.5) appropriately. Nonetheless, we acknowledge that the derived prediction capability of GMI is specific to the patient cohort in this study and may be subject to influences by the lower GMI-positive prevalence rates here. Of note, we have also performed similar analyses of GMI trends with patients with just-proven and probable IA in this cohort ($n = 147$) and obtained results similar to those described above. While being a retrospective analysis, the strength of this study lies in that it utilizes the largest cohort of IA patients involved in a major, well-conducted, multicenter clinical trial where the case definitions, endpoints, and criteria for the determination of a clinical response were well established and objectively determined by a blinded panel of experts.

In conclusion, we have shown that early GMI trends can be used to help predict eventual clinical responses and mortality for patients with IA. The early identification of patients who may potentially fail therapy is crucial for facilitating prompt interventions and optimizing outcomes of invasive aspergillosis.

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